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David Fichtenberg
Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules
PO Box 7577
Olympia, WA 98507-7577

July 7, 1997

The Secretary
Federal Communications Commission
1919 M. Street N.W. Room 222
Washington, DC 20554

In the Matter of	)	ET-Docket No. 93-62
	)	and in this docket pertaining to:
Guidelines for Evaluating the Environmental	)	- Report and Order FCC 96-326
Effects of Radiofrequency Radiation	)	- First Memorandum ofUnderstanding Order FCC 96-487

Ex Parte Comments Pertaining to ET-Docket 93-62 Regarding
PETITIONS FOR RECONSIDERATION of Commission Rule & Order FCC 96-326, and First Memorandum of Opinion and Order FCC 96-487

with original and 2 copies submitted to the Secretary of the Commission
in accordance with 47 CFR §1.1202, 1.1203, and 1.1206(a)
3rd Ex Parte Submission

Dear Mr. Secretary,

Enclosed please find an original and 1 copies of an ex parte presentation pertaining to ET-Docket 93-62 and being submitted in accordance with 47 CFR §1.1202, 1.1203, and 1.1206(a). Please assure these are put in the official record of this proceeding.

The presentation includes comments and copies of footnoted papers including a pre-publication copy (with author's corrections) of footnote #3 - "Melatonin and a Spin-Trap Compound Block Radio-frequency DNA Strand Breaks in Rat Brain Cells," by Henry Lai and Narendra P. Singh, to be published in 1997 in Bioelectromagnetics, Vol. 18.

Thank you,

David Fichtenberg

David Fichtenberg
Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules
PO Box 7577
Olympia, WA 98507-7577 Tel: (206) 722-8306

Copies sent to list on the following page.

Copies sent to:

Chairman Reed E. Hundt
Federal Communications Commission
1919 M Street, N.W. Room 814
Washington, D.C. 20554

Commissioner James H. Quello
Federal Communications Commission
1919 M Street, N.W. Room 802
Washington D.C. 20554

Commissioner Rachelle B. Chong
Federal Communications Commission
1919 M Street, N.W. Room 844
Washington, D.C. 20554

Commissioner Susan Ness
Federal Communications Commission
1919 M Street, N.W. Room 832
Washington D.C. 20554

Mr. Robert J. Cleveland, Jr.
Office of Engineering and Technology
Federal Communications Commission
2000 M Street N.W. Room 480
Washington, D.C. 20554

Mr. David Wye
Wireless Telcommunications Bureau
Federal Communications Commission
2025 M Street N.W. Room 5002
Washington, D.C. 20554

Before the

FEDERAL COMMUNICATIONS COMMISSION

Washington, DC 20554

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To: The Commission

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To: The Commission

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in accordance with 47 CFR §1.1202, 1.1203, and 1.1206(a)

Submitted by the Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules, PO Box 7577, Olympia, WA 98507-7577

1. Introduction:

1.1 Appropriate submission of an ex parte presentation

The Ad-hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency ("RF") Health and Safety Rules ("the Ad-Hoc Association") understands (i) that a Federal Communications Commission ("Commission") "Sunshine Agenda" period per 47 CFR §1.1202(f) and §1.1203 is not now in effect regarding ET-Docket 93-62; (ii) that administrative finality has not yet been decided upon concerning the Commission's responses to Petitions For Reconsideration that have been submitted in this proceeding; and that (iii) this proceeding permits ex parte presentations in accordance with 47 CFR §1.1202, 1.1203, and 1.1206(a), and in accordance with the April 8, 1993 Notice of Proposed Rule Making in ET-Docket 93-62, paragraph 30. Accordingly, the Ad-Hoc Association is properly making this ex parte submission.

1.2. Purpose of presentation is to provide not yet published documentation evidence which was referenced by the Ad-Hoc Association further evidence which (i) supports its requests and its claims in its petition for reconsideration regarding FCC 96-326 and dated September 6, 1991 ("Ad-Hoc 96-326 Petition") and its petition for reconsideration regarding FCC96-487 and dated February 21, 1997 ("Ad-Hoc 96-487 Petition"), (ii) and which supports its evidence and claims in its ex parte written presentation dated June 10, 1997 ("Ad-Hoc June 10 submission") and its ex parte written submission dated June 30, 1997 ("Ad-Hoc June 30 submission"), and also to provide additional evidence. To the extent that these comments rely on findings that were not previously presented to the Commission, these facts and reports became available after the last opportunity for filing in this matter, excluding ex parte presentations, and in any event, consideration of these facts and comments significantly relates to changes needed for the public health and is in the public interest. In this way, the Ad-Hoc Association is providing an opportunity for the Commission to review and pass upon the matters presented herein[1], and by so doing the Commission will have the opportunity of considering any newly discovered evidence[1], and the Commission will also thus have the opportunity of reviewing objections not first raised previously[1] or clarifications and which support the requests in the Ad-Hoc Association FCC 96-326 and FCC 96-487 petitions, and in any event, even if the Commission find otherwise, the Commission's consideration and approval of Ad-Hoc Association requests is in the public interest. Should the Commission find it should make changes elsewhere in its rules based on the evidence herein, it is requested that it do so, and make any other modifications it finds to be just and proper to serve the public interest.

2. Documentation that RF directly causes increases in free radicals and which supports more restrictive limits

2.1 Evidence for direct RF causation of increased free radicals in the brain

2.1.1 Request in Ad-Hoc 96-326 petition:

The Ad-Hoc 96-326 Petition explicitly noted adverse effects to the brain of rats exposed to RF and requested the Commission identify an average whole body SAR of 0.0008 W/kg as a hazard threshold to which to apply the traditional uncertainty factor of 100, resulting in a 'safe' level of an

average whole body SAR = 0.000008 W/kg, 1/10,000th of present average while body limits of 0.08 W/kg upon which the Commission's power density limits derive.[see Ad-Hoc 96-326 Petition item #19, 19.1-19.3 at page 15-16, and Ad-Hoc June 30 submission item #1.3.7-1.3.12 at page 1.4-1.7. Allowing for the Commission's limit for increase in SAR for a localized part of the body to be 20 fold more than the average, indicates the Ad-Hoc 96-326 Petition requesting local SAR of the head to be no more than 0.00016 W/kg.

2.1.2 Increase in free radicals found by H. Lai and N.P. Singh[2,3]

As noted in the Ad-Hoc June 30 submission, additional evidence for keeping exposure as low to the brain, is that recent evidence from Lai et al. (1997)[2,3] suggests that free radicals are created in the brain due to RF at 0.6 W/kg, and that this is a direct action of the RF exposure on the brain. H. Lai announced at a Food and Drug Administration Workshop on February 7, 1997[216] that he was able to replicate previous findings reporting increased single and double strand DNA breaks when exposed to RF at 2450 MHz 2 microsecond pulse duration and 500 pulses per second. He also found that the affect disappeared when the rats were injected with either melatonin or another compound known to be a free-radical scavenger. The effect was clear and dramatic. Control animals not handled, sham controls, and RFR exposed treated with either melatonin or PBN had almost identical distributions of DNA migration length. In contrast, the distribution of cells treater with RFR and receiving only a saline vehicle solution (and not melatonin or PBN) had a dramatic increase shifting the distribution of DNA migration length to much longer values, indicating more DNA breaks (see enclosed article).

The authors report,

"Melatonin has been reported to be a free radical scavenger. It has been shown to inhibit DNA-adduct formation induced by the carcinogen safrole in vivo and to protect lymphocytes from radication-induced chromosome damage in vitro. In addition, an advantage of using melatonin in this study is that it can readily pass through the blood-brain barrier and cell and nuclear membranes. PBN has been shown to protect cells from free radical-induced apoptosis. In particular, various studies have reported that PBN can reverse free radical-related damage to the nervous system. For example, it has been shown to reverse age-related changes in protein chemistry in the brain and deterioration in spatial memory functions in the rat."

"Data from the present experiment confirm our previous finding that acute RFR (radiofrequency radiation) exposure causes an increase in DNA single- and double-strand

breaks in brain cells of the rat. In addition, we have found that the effect can be blocked by treating animals with melatonin or PBN (N-tert-butyl-α-phenylnitrone). Since a common property of melatonin and spin-trap compounds (such as PBN) is that they are efficient radical scavengers, these data suggest that free radical may play a role in RFR-induced DNA single- and double-strand breaks observed in the brain cells of the rat."

"Consistent with this hypothesis is the fact that free radicals can cause damage to DNA and other macro-molecules in cells. Particularly, oxygen free radicals have been known to cause DNA strand breaks. In addition, a study has implicated free radicals as the cause of some of the biological effects observed after exposure to RFR. Phelan et al [1992][4] reported that RFR can interact with melanin containing cells and lead to changes in membrane fluidity consistent with a free radical effect. [note: the effect disappeared when a substance that readily combines with free radicals was injected into the animals to scavenge for free radicals].

"If free radicals are involved in the RFR-induced DNA strand breaks in brain cells, results from the present study could have an important implication on the health effects of RFR exposure. Involvement of free radicals in human diseases, such as cancer and atherosclerosis, have been suggested. Free radicals also play an important role in aging processes. Aging has been ascribed to accumulated oxidative damage to body tissues, and involvement of free radicals in neurogenerative diseases, such as Alzheimer's Huntington's and Parkinson's has also been suggested."

"Since both melatonin and PBN are efficient free radical scavengers, it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and since an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure.[3]" [for references on other study findings reviewed by Lai and Singh see footnote 3]

Moreover, since free radicals have a very short life this suggests they are generated close to sites where the DNA has been damaged. Thus, it is reasonable to conclude this is a direct RF effect on the brain, and hence that RF exposure to the brain should be kept as low as possible.

The exposure was at 2 mW/sq. cm with an average SAR of 1.2 W/kg; based on previous studies[5] Lai et al. report that the SAR in the brain range from 0.5 to 2 W/kg per 1 mW/cm2 irradiation in their exposure system. Therefore for the present study the SAR variation in the brain would be here 1 to 4 W/kg. [Note: in the Ad-Hoc June 30 submission on page 97, line 14, where it states, "based on previous studies218 Lai et al. report ....." , instead of footnote 218 it should be footnote 31 in that ex parte submission, which in this ex parte submission is footnote 5].

Since an actual increase in the concentration of free radicals resulted, a lower value would seem appropriate for a threshold, say , 1/2 to 1/10th the value at which the present effect was

observed (i.e. 0.1 to .4 using a 1/10th uncertainty factor to obtain a threshold vs. 0.5 to 2 W/kg using 1/2 uncertainty factor to obtain a threshold.. Then applying a 'safety factor of 100 yields 0.001 W/kg to 0.02 W/kg as SAR limits.

These low exposure values are much lower than the 8 W/kg of exposure allowed for worker exposure in any local part of the body, e.g. the head. Since, it is assumed in the 1986 National Council of Radiation Protection and Measurement RF standard and the RF standard of the Institute of Electrical and Electronic Engineers (IEEE), IEEE C95.1-1991 that exposure to the head may be as high as 20 fold the average whole body SAR, this implies that the average whole body SAR should not be greater than 0.001/20 = 0.00005 W/kg.

2.1.3 The study by Lai and Singh have two important implications:

- It dramatically shows that at a whole body average SAR of 1.2 W/kg that adverse effects (free radical generation) occurs.

- It provides strong evidence that the effect is direct on the brain, and therefore, that local SAR at the brain must be below 1.2 W/kg with an appropriate uncertainty factor (as shown in 2.1.2 above) to apply for a 'working threshold' and chronic effects, species effects, and special needs of some human populations. Hence, the levels deemed "safe" (1.6 W/kg) for the general population, are too high since, given the above study and continuing uncertainties, this limit does not demonstrate sufficient caution for being protective.

2.2 Increase in free radicals found by Phelan et al.[8] [and noted in Ad-Hoc June 30 submission at item 2.2 at page 4, and at footnote 5 therein].

It is a very significant finding that RF at 0.2 W/kg and at 2450 MHz provides evidence for melanin tissue of the skin and eyes causing free radicals[8]. First, this finding supports the finding of evidence for an increase in free radicals in the brain due to RF [see Lai and Singh at 2.1 above]. Moreover, it justifies further the claims and requests of the Ad-Hoc FCC 96.326 petition that the 4 W/kg hazard threshold of the Commission needs to be lower. While noted at length in the Ad-Hoc June 30 submission, it is noted again here insofar as this ex parte comment focuses on RF induced increases in free radicals and other potential adverse effects on chromosomes and other molecules.

3. Chromosomal damage or change due to RF: The above evidence that RF causes free radicals, which in turn can cause chromosome aberrations (as noted by Lai and Singh), is consistent with evidence of chromosomal changes at low intensities of RF - and which is also independent evidence that the Commission's limits are not sufficiently stringent.

3.1 At 1 microwatt /sq. cm at 41.32 GHz and X-ray radiation at 20 Gy the *"irradiation for 5 minutes significantly suppressed genome conformal state repair* (i.e. repair of a chromosome to its natural shape) in comparison to only X-ray radiation., the SAR was about 0.0073 W/kg[6] [and referenced in Ad-Hoc FCC 96-326 Petition at page 16 and footnote 107 therein].

3.2 Epigenetic effects[7] [referenced in Ad-Hoc 96-326 Petition at footnote 121] have included:

- In vitro for E.coli:

3.2.1 At 42.25 GHz, 46.15 GHz, and 51.75 GHz at 0.001 to 1 mW/sq. cm "induction of colicine synthesis." [ref. #2 in footnote 7]

3.2.2 At 51.3 to 52.2 GHz and at 0.5 mW/sq. cm colicine was affected. [ref. #5 in footnote 7]

3.2.3 At 70.3 to 70.5 GHz with resonance at 70.4 GHz and at 0.2 to 0.5 mW/sq. cm. there was prophage induction. [ref #.1 in footnote 7]

3.2.4 At 40 to 60 GHz with resonance at 41.268 GHz and at 0.1 to 0.2 mW/sq. cm. there was prophage induction [ref. #6 in footnote 7]

3.2.5 At 70.3 to 70.6 GHz with resonance at 70.37 GHz and at 0.1 to 0.5 mW/sq. cm there was prophage induction reported by other researchers. [ref. #11 in footnote 7]

- in vitro for higher eukaryotes (cells with a nucleus):

3.2.6 At 64.1 to 69.1 and at 5 mW/sq. cm. there was *"regression of the BR2 puff of polytene chromosomes, reduction of RNA synthesis and protein secretion."* [ref. #12 in footnote 7]

3.2.7 At 41.67 GHz and at 5 mW/sq. cm. there was significant induction of somatic mosaicism." [ref #13 in footnote 7]

3.2.8. At 46.0 to 46.5 GHz with resonance at 46.35 and at 0.1 mW/sq. cm there was *"increased level of morphoses."* [ref. #14 in footnote #7]

3.2.9. At 41.01 to 42.96 GHz at 0.02 to 2.5 mW/sq. cm there was found *"seven-fold increase in centromeric decondensation of A chromosomes."* [ref. #15 in footnote 7]

- In vivo direct and indirect indications of non-mutagenic action of RF:

3.2.10 At 41.67 GHz and at 5 mW/sq. cm. "induction of somatic mosaicism without dominant lethal mutations" [ref. #13 in footnote 7]

3.2.11 At 37.46 to 78.40 and at 0.02 to 10 mW/sq. cm. in this range found "frequency-dependent decondensation of chromatin without aberrations of chromosomes." [ref #15 in footnote 7]

3.2.12 At 70.3 to 70.6 GHz at 0.5 mW/sq. cm. *"changed conformal state of genome without cell death (no DNA changes)."* [ref. #19 in footnote 7]

3.3 At 10 to 50 microwatts /sq. cm. radar workers exposed to microwaves had increased chromosome abberations[8]. 10 radar station workers and 10 from a polyvinylchloride industry were randomly chosen, and were employed from 8 to 25 years. Persons who had not been occupationally exposed to any known mutagenic agent were chosen as controls.

4. At a Food and Drug Administration (FDA) February1997 seminar, E.Czerska of the FDA reported new findings[4b] of statistically significant increases in cell proliferation of human glioblastoma tumor cells after 24 hours of exposure to 827 MHz modulated like some cellular phones, and at an SAR of 1.6 W/kg[4b], the limit for the public for exposure to the head, and 1/5th of the 8 W/kg limit for workers. This finding further supports Ad-Hoc Association claims.

Footnotes:

1. (i) Action For Children's Television v FCC, 564 F.2d 458,468,469 (1977);
(ii) Joseph v. FCC, 131 U.S. App.D.C. 207,210, 404 F.2d.207,210(1968);
(iii) Gerico Investment Co. v. FCC, 99 U.S. App.D.C. 379,380, 240 F.2d 410,411 (1957); (iv) Saginaw Broadcasting Co. v. FCC, 99 U.S. App. D.C. 282, 286, 96 F.2d. 554, 558, 59 S.Ct. 72, 83 L.Ed. 391 (1938) and other citations at Action For Children's Television v FCC at pg 469.

2. H. Lai announcement at FDA February meeting that melatonin blocks RF DNA strand break effect is reported in "FDA Workshop on Biological Effects of Wireless Radiation: Politics and Lack of Research Funds Stymie Progress," in Microwave News March/April 1997, pg. 9 and abstracts are available at the Microwave Web site: www.microwavenews.com.

3. H.Lai and N.P. Singh, "Melatonin and a Spin-Trap Compound Block Radiofrequency Electromagnetic Radiation-Induced DNA Strand Breaks In Rat Brain Cells," Biolectromagnetics, 1997: Vol. 18. in press. - prepublication copies available to the Commission upon request.

4. A. Phelan, et al. "Modification of Membrane Fluidity in Melanin-Containing Cells by Low-Level Microwave Radiation," Bioelectromagnetics, Vol. 13, pages 131-146, (1992).

4b. E.Czerska et al, "Effects of Radiofrequency Electromagnetic Radiation on Cell Proliferation," reported at FDA workshop "Physical Characteristics and Possible Biological Effects of Microwaves as Applied to Wireless Communication," Feb. 7, 1997, Rockville, Md.

5. Chou et al. (1985), "Specific absorption rate in rats exposed to 2450 MHz microwaves under seven exposure conditions," Bioelectromagnetics, Vol: 6: pg. 73-88.

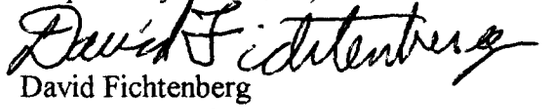
6. I.Y. Belyaev, "Resonance Effect of Microwave on the Genome Conformal State of E.coli Cells," in English and published in Zeitschrift fur Naturforschung, Section C, J.Bioscience 47c:621-627, 1992, and referenced in Bioelectromagnetics17:166 (1996).
7. I.Ya.Belyaev, "Some biophysical aspects of the genetic effect of low-intensity millimeter waves," Biochemistry and Bioenergetics, 27:11-18, 1992
8. V. Geraj-Vrhovac et al., "Comparison of chromosome aberration and microneucleus induction in human lymphocytes after occupational exposure to vinyl chloride monomer and microwave radiation," Periodicum Biologorum, Vol. 92, No. 4, pg. 411-416, (1990)

In this **ex parte** submission the Ad-Association has provided pre-publication documentation of peer-reviewed research showing that levels of RF (1.2 W/kg) deemed save for exposing workers (at up to 8 W/kg) and the general public (at up to 1.6 W/kg) can create an increase in free radicals in the brain. This evidence, the evidence that RF at 0.2 W/kg can increase free radicals in the melanin containing tissues of the skin and eye, and the evidence that at 'safe' limits for millimeter length waves there are epigenetic and related effects, provides strong support for making the Commission's limits more stringent. Copies of pertinent papers footnoted above (#2 to #7) have been included with this submission.

Since (i) the Commission has stated it does not have the expertise to evaluate the evidence and claims herein, (ii) the Commission stated it defers to the federal health agencies which have participated in this proceeding, and (iii) the Commission does have responsibility for appropriately setting its exposure criteria, therefore the Commission should ask aforementioned federal health agencies to evaluate the Ad-Hoc Association evidence and claims as they pertain to RF biologic and health related matters. Please contact the Ad-Hoc Association if the Commission or any parties reviewing this document are unable to find referenced materials or have questions.

Signature:

Respectfully Submitted,



David Fichtenberg Dated: July 7, 1997
Spokesperson for the Ad-Hoc Association of Parties Concerned About the Federal Communications Commission's Radiofrequency Health and Safety Rules et al
P.O. Box 7577

Olympia, WA 98507-7577 Tel: (206) 722-8306

FDA Workshop on Biological Effects of Wireless Radiation: Politics and Lack of Research Funds Stymie Progress

The most intriguing finding presented at the February Food and Drug Administration (FDA) workshop* on wireless radiation was 30 years old. In a talk on "Headaches from Cell Phones: Are They Real?" Dr. Allan Frey reported that in the 1960s, while he was studying microwave hearing, a number of his subjects complained about headaches. "I was sufficiently concerned about the headaches to stop research with humans," said Frey, who is credited with discovering microwave hearing.

To make his point, Frey, a researcher and consultant based in Potomac, MD, distributed a paper presented at an FDA symposium in 1969, in which he had written:

> ...I noticed that headaches appeared to be induced at some frequencies at low power levels. A limited amount of exploration leads me to believe that the headache effect is probably real, but it requires verification. Thus, it is mentioned here only as a hypothesis for research.†

So far, no one has tested Frey's hypothesis. But he remains convinced that cellular phones can cause headaches because, as he reminded the audience, "You get penetration into the head at those frequencies." And his concerns run deeper. "Headaches may only be the most obvious indicator of what is going on biologically," he warned. Frey believes that the headaches may stem from microwave-induced leakage through the blood-brain barrier.

The FDA workshop was held four years after the Cellular Telecommunications Industry Association (CTIA) launched its five-year research program designed to show that its products are safe (see *MWN*, J/F93).

Wireless Technology Research (WTR), created by the CTIA to run the program, has yet to sponsor a single biological experiment. The program is now at a standstill due to concerns over legal liability (see p.8 and *MWN*, M/J96). Some at the FDA workshop were openly skeptical that any biological studies would emerge from the industry effort.

"It is ultimately frustrating that no one wants to fund this research," complained Dr. Stephen Cleary of Virginia Commonwealth University in Richmond at the end of his talk on *in vitro*, nonthermal effects.

Cleary's concerns were echoed by another veteran microwave researcher, Dr. Henry Lai of the University of Washington, Seattle. Lai and his collaborator, Dr. N.P. Singh, have been unable to find support to continue their studies. Though long promised WTR research funds, they are still empty-handed. "I am disappointed," Lai told *Microwave News*. "We have been waiting for money for more than two years."

The FDA's own contribution has been minimal. The agency has opted to watch WTR's effort from the sidelines—with a few exceptions. Howard Bassen of the FDA's Center for Devices and Radiological Health (CDRH) has been evaluating probes to measure RF electric fields and methods of estimating energy deposition in the brain.

And the CDRH's Dr. Ewa Czerska is attempting to replicate Cleary's experiments showing that RF/MW radiation can enhance the proliferation of human brain tumor cells (see *MWN*, M/A90). In contrast to Cleary, who used 27 MHz and 2450 MHz radiation, Czerska is using 827 MHz radiation signals designed to mimic the emissions from a digital cellular phone. Czerska announced that she had at least partial confirmation of Cleary's results, observing greater proliferation at specific absorption rates of 1.6 W/Kg and 4.8 W/Kg. "The increase also appeared to be dose-dependent," she said. Czerska noted that this could not have been due solely to a thermal response, since conventional heating did not stimulate a similar level of proliferation.

Lai's latest results, first publicly announced at the FDA workshop, add a new twist to the microwave–DNA story. He reported that the previously observed increases in DNA single- and double-strand breaks are blocked by melatonin.

The political dimension of cellular phone research came into focus with the presentations by Drs. Ross Adey of the VA Hospital in Loma Linda, CA, and Joseph Roti Roti of Washington University in St. Louis.

Adey reviewed the results of his long-term exposure study for Motorola, which indicated that digital (TDMA) cellular phone signals had a protective effect against brain tumor development in rats (see *MWN*, J/A96). But Adey has yet to publicly disclose that a second, parallel exposure using continuous-wave, frequency-modulated (FM) microwaves had absolutely no effect (see p.13).

The biological impacts of FM and TDMA radiation are quite different, Adey said, adding that he could not elaborate further because, "I have been interdicted from talking about FM." The take-home lesson, said Adey, is that, "Every signal may have a different effect."

Roti Roti said that he had not observed the DNA breaks reported by Lai and Singh. But he noted that he had used a different type of microwave radiation and an *in vitro* assay rather than live rats.

At that point, a heated discussion ensued as to whether Roti Roti had used an appropriate signal. "I did not make the decision about the signal," an exasperated Roti Roti finally exclaimed. "I did not pick it. Talk to the lawyers who wrote the contract."

Motorola had a lawyer as well as a public relations consultant from Burson-Marsteller at the workshop. The lawyer, Charles Eger, who had also been in Victoria, Canada, for Adey's presentation of the Motorola TDMA rat study last June, would not say who had picked the experimental conditions for Roti Roti's study. "I'm not familiar with the contract," Eger told *Microwave News*. "I'm not a practicing lawyer; I'm a policy guy."

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* *Physical Characteristics and Possible Biological Effects of Microwaves as Applied to Wireless Communication*, held at the FDA, Rockville, MD, February 7, 1997. The abstracts of the talks are available at the *Microwave News* Web site, <www. microwavenews.com>.

† Allan Frey, "Effects of Microwave and Radiofrequency Energy on the Central Nervous System," *Biological Effects and Health Implications of Microwave Radiation: Symposium Proceedings*, Richmond, VA, September 17-19, 1969, pp.134-139.

(In Press)
Bioelectromagnetics 00:000–000 (1996)

Melatonin and a Spin-Trap Compound Block Radiofrequency Electromagnetic Radiation-Induced DNA Strand Breaks in Rat Brain Cells

Henry Lai* and Narendra P. Singh

Bioelectromagnetics Research Laboratory, Center for Bioengineering, University of Washington, Seattle, Washington

Effects of in vivo microwave exposure on DNA strand breaks, a form of DNA damage, were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to pulsed (2 μsec pulses, 500 pps) 2450-MHz radiofrequency electromagnetic radiation (RFR) (power density 2 mW/cm^2, average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-α-phenylnitrone (PBN) (100 mg/kg/injection, IP) blocks this effect of RFR. Since both melatonin and PBN are efficient free radical scavengers, it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and since an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure. *Bioelectromagnetics 18:000–000, 1997.* © 1997 Wiley-Liss, Inc.

Key words: radiofrequency electromagnetic radiation (RER); brain cells; DNA single- and double-strand breaks; melatonin; N-tert-butyl-α-phenylnitrone (PNB); free radicals

INTRODUCTION

Recently, we reported an increase in DNA single- and double-strand breaks in the brain cells of rats exposed for 2 hours to pulsed 2450-MHz radiofrequency electromagnetic radiation (RFR) at averaged whole body specific absorption rates (SAR) of 0.6 and 1.2 W/kg [Lai and Singh, 1995, 1996]. In these experiments, DNA strand breaks were assayed 4 hours post exposure.

The mechanism by which RFR causes this effect is not known. In the present study, we investigated whether free radicals play a role. Rats were treated with the free radical scavengers melatonin and N-tert-butyl-α-phenylnitrone (PBN) to investigate whether they can block RFR-induced DNA single- and double-strand breaks in brain cells. Melatonin has been reported to be a free radical scavenger [Reiter et al., 1995]. It has been shown to inhibit DNA-adduct formation induced by the carcinogen safrole in vivo [Tan et al., 1993] and to protect lymphocytes from radiation-induced chromosome damage in vitro [Vijayalaxmi, 1995]. In addition, an advantage of using melatonin in this study is that it can readily pass through the blood-brain barrier and cell and nuclear membranes [Costa et al, 1995; Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al, 1993]. PBN has been shown to protect cells from free radical-induced apoptosis [Slater et al., 1995]. In particular, various studies have reported that PBN can reverse free radical-related damage to the nervous system. For example, it has been shown to

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*Correspondence to: Henry Lai, Ph.D., Center for Bioengineering, Box 357962, University of Washington, Seattle, WA 98195.

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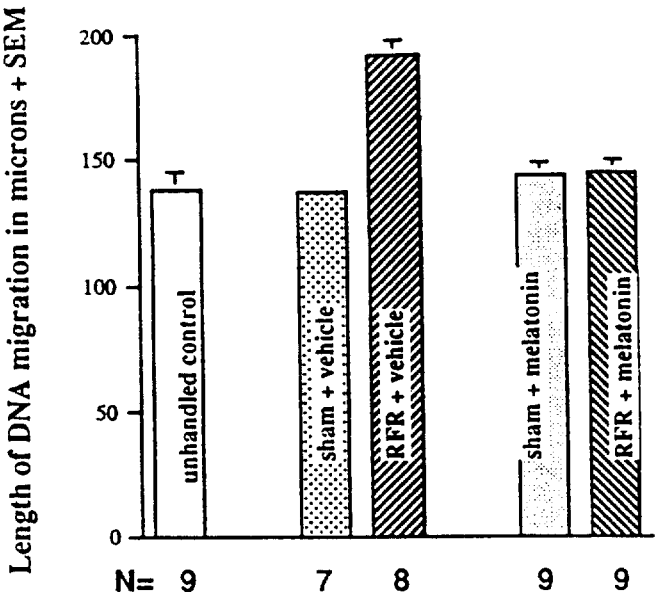

Fig. 1. Effect of treatment with melatonin on RFR-induced increase in DNA single-strand breaks in rat brain cells. Data was analyzed using the one-way ANOVA, which showed a significant treatment effect ($F[4,37] = 16.59$, $P < .001$).

reverse age-related changes in protein chemistry in the brain and deterioration in spatial memory functions in the rat [Carney and Floyd, 1991; Carney et al., 1991]. It can reverse ischemia-induced free radical injury in the brain [Oliver et al., 1990], inhibit free radical release after experimental brain concussion [Sen et al., 1991], and reduce infarct size in the brain following transient middle cerebral artery occlusion [Zhao et al., 1994].

METHODS AND PROCEDURES

Animals

Male Sprague-Dawley rats (250-300 g) purchased from B & K Laboratory, Bellevue, WA, were used in this research. They were housed three to a cage in a room adjacent to the RFR exposure room for 48 hours before an experiment. The laboratory was maintained on a 12-hour light-dark cycle (light on 6:00–18:00 h) and at an ambient temperature of 22 °C and a relative humidity of 65%. Animals were given food and water ad libitum.

RFR Exposure System and Exposure Conditions

The cylindrical waveguide system developed by Guy et al. [1979] was used for RFR exposure. The system consists of individual cylindrical waveguide tubes connected through a power divider network to a single RFR power source. Each tube consists of a section of circular waveguide constructed of galvanized wire screen in which a circularly polarized TE_{11} mode field configuration is excited. The tube contains a plastic chamber that houses a rat with enough space to allow free motion. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside the waveguide. Waveguides were calibrated and checked from time to time.

This waveguide system, using circularly polarized radiation, enables efficient coupling of radiation energy to the animal exposed. For example, a spatially averaged power density of 1 mW/cm^2 in the circular waveguide produces a whole-body SAR of 0.6 W/kg in the rat [Chou et al., 1984]. The range of power densities for exposure to a linearly polarized plane-wave associated with an SAR of 0.6 W/kg is approximately 3–6 mW/cm^2. By connecting this system to a pulsed signal source (Applied Microwave, model PG5KB), rats were irradiated with pulsed (2 μsec pulse width, 500 pulses per second) 2450-MHz radiation at a spatially averaged power density of 2 mW/cm^2, which gave an averaged whole-body SAR of 1.2 W/kg. Since each waveguide can be activated individually, an animal can be subjected to either RFR- or sham-exposure in a waveguide. Both RFR- and sham-exposed animals were included in each exposure session.

In the experiment, animals were injected with melatonin (Sigma Chemical Co., St. Louis, MO; 1 mg/kg/injection, SC, dissolved in a concentration of 1 mg/ml in 1% ethanol-saline solution) or an equal volume of its vehicle, or with N-tert-butyl-α-phenylnitrone (PBN) (Sigma Chemical Co., St. Louis, MO; 100 mg/kg/injection, IP, dissolved at 25 mg/ml in physiological saline) or an equal volume of its vehicle. Injections were given immediately before and after exposure. The drug dosages used were based on previous studies showing efficient free radical scavenging effects, especially in the brain [Carney et al., 1991; Chen et al., 1994; Kothari et al., 1995; Lafon-Cazal et al., 1993a,b; Melchiorri et al., 1995; Tan et al., 1993; Zhao et al., 1994]. Melatonin and PBN solutions were prepared immediately before injection, and exposure to light and air were kept at a minimum. Since the drugs have a short half-life (0.5–2 hours) in the blood, the experimental schedule involved two hours of exposure and four hours of post-exposure waiting, and the exact time when DNA strand breaks occurred was not known, we decided to inject the animals twice: before and after exposure.

Therefore, there were four treatment groups for each drug (melatonin and PBN)-treatment experiment: RFR/drug; RFR/vehicle; sham/drug; and sham/vehicle. In addition, a group of unhandled animals was included

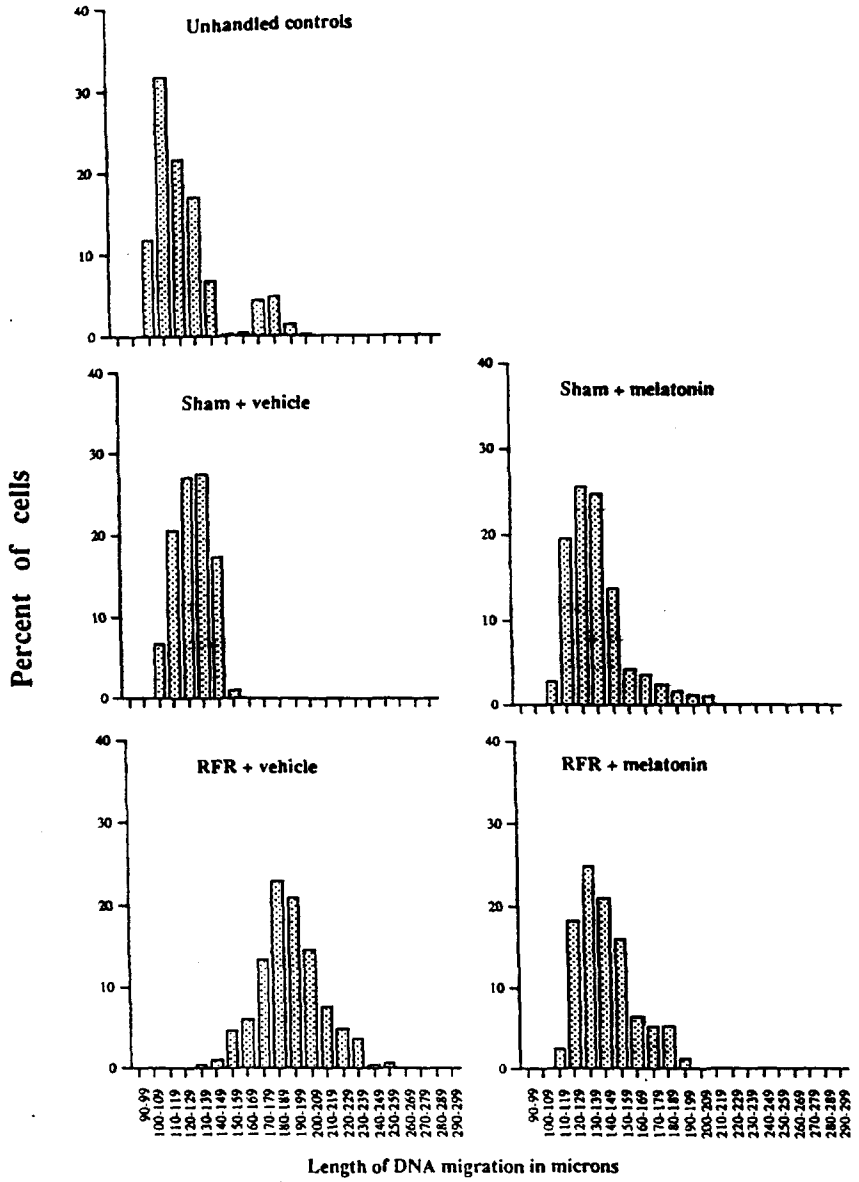

Fig. 2. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 1.

in each experiment. These animals were housed in their home cage for the entire period of the experiment, and DNA strand breaks were assayed in their brains without experimental treatment and handling. These animals controlled for the possible effect of experimental procedures on DNA strand breaks in brain cells.

The animals were returned to their home cages after exposure. Four hours later, each rat was placed for 60 seconds in a closed foam box containing Dry Ice (a cardboard was put on top of the Dry Ice to prevent its direct contact with the animal) and then decapitated with a small animal guillotine. Dry Ice was used in the euthanasia because its use minimizes red blood cell contamination of tissue samples which could affect DNA strand break measurements. All procedures from this step onward were done in minimum indirect light. Brains were immediately dissected out from the skull for assay of DNA strand breaks. Dissection of a brain took approximately 30 seconds.

All experiments were run blind. The on/off conditions of the waveguides were determined by an experimenter before an experiment. Two other experimenters, who did the animal exposure/brain dissection and DNA strand-break assay, respectively, did not know

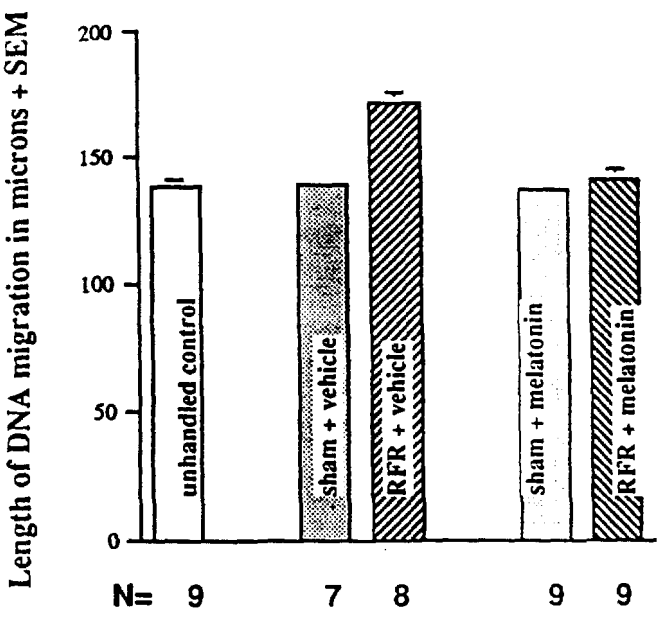

Fig. 3. Effect of treatment with melatonin on RFR-induced increase in DNA double-strand breaks in rat brain cells. One-way ANOVA of the data showed a significant treatment effect ($F[4,37] = 19.02$, $P < .001$).

the treatment conditions (RFR or sham exposure) of the rats.

Assay Methods for DNA Strand Breaks

The microgel electrophoresis assay for DNA single- and double-strand breaks in rat brain cells was carried out as described previously in Lai and Singh [1996]. All chemicals used in the assay were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise noted. Immediately after dissection, a brain was immersed in ice-cold phosphate-buffered saline (PBS) (NaCl, 8.01 g; KCl, 0.20 g; Na_2HPO_4, 1.15 g; KH_2PO_4, 0.20 g, per liter, pH 7.4) containing 200 μM of N-t-butyl-α-phenylnitrone. The tissue was quickly washed four times with the PBS to remove most of the red blood cells. A pair of sharp scissors was used to mince (approximately 200 cuts) the tissue in a 50-ml polypropylene centrifuge tube containing 5 ml of ice-cold PBS to obtain pieces of approximately 1 mm^3. Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 ml of PBS, tissue pieces were dispersed into single-cell suspensions using a P-5000 Pipetman. This cell suspension consisted of different types of brain cells. Ten microliters of this cell suspension were mixed with 0.2 ml of 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 37 °C, and 30 μl of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24 × 50 mm square #1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed and 200 μl of agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium N-lauroyl sacosinate, 100 mM disodium EDTA, 10 mM Tris base, pH 10) containing 1% Triton X-100.

To measure single strand DNA breaks, after lysing overnight at 4 °C, slides were treated with DNAase-free proteinase K (Boehringer Mannheim Corp., Indianapolis, IN) in the lysing solution for 2 hours at 37 °C. They were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA) modified so that both ends of each electrode were connected to the power supply. One liter of an electrophoresis buffer (300 mM NaOH, 0.1% of 8-hydroxyquinoline, 2% dimethyl sulfoxide, and 10 mM tetra-sodium EDTA, pH 13) was gently poured into the assembly to cover the slides to a height of 6.5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0.4 volt/cm, approximately 250 mA, for 60 min) and the buffer was recirculated.

At the end of the electrophoresis, electrophoretic buffer above the slides was gently removed. Slides were then removed from the electrophoresis apparatus and immersed in neutralization buffer (0.4 M Tris at pH 7.4) in a Coplin jar (two slides per jar) for 10 min. After two more similar steps of neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar for 30 min and then dried.

For double-strand breaks, microgel preparation and cell lysis were done as mentioned above. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp., Indianapolis, IN) (10 μg/ml in the lysing solution) for 2 hours and then with proteinase K (1 mg/ml in the lysing solution) for 2 hours at 37 °C. They were then placed for 20 min in an electrophoretic buffer (100 mM Tris, 300 mM sodium acetate and acetic acid at pH 9.0), and then electrophoresed for 1 hour at 0.4 volt/cm (approximately 100 mA). The slides were treated with 300 mM NaOH for 10 min and neutralized as before with 0.4 M Tris (pH 7.4). Slides were then dehydrated in absolute ethanol for 30 min and dried.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was taken out and stained with 50 μl of 1 μM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50-mm coverglass. Slides were examined and analyzed with a Reichert vertical fluorescent microscope (model 2071) equipped with a filter com-

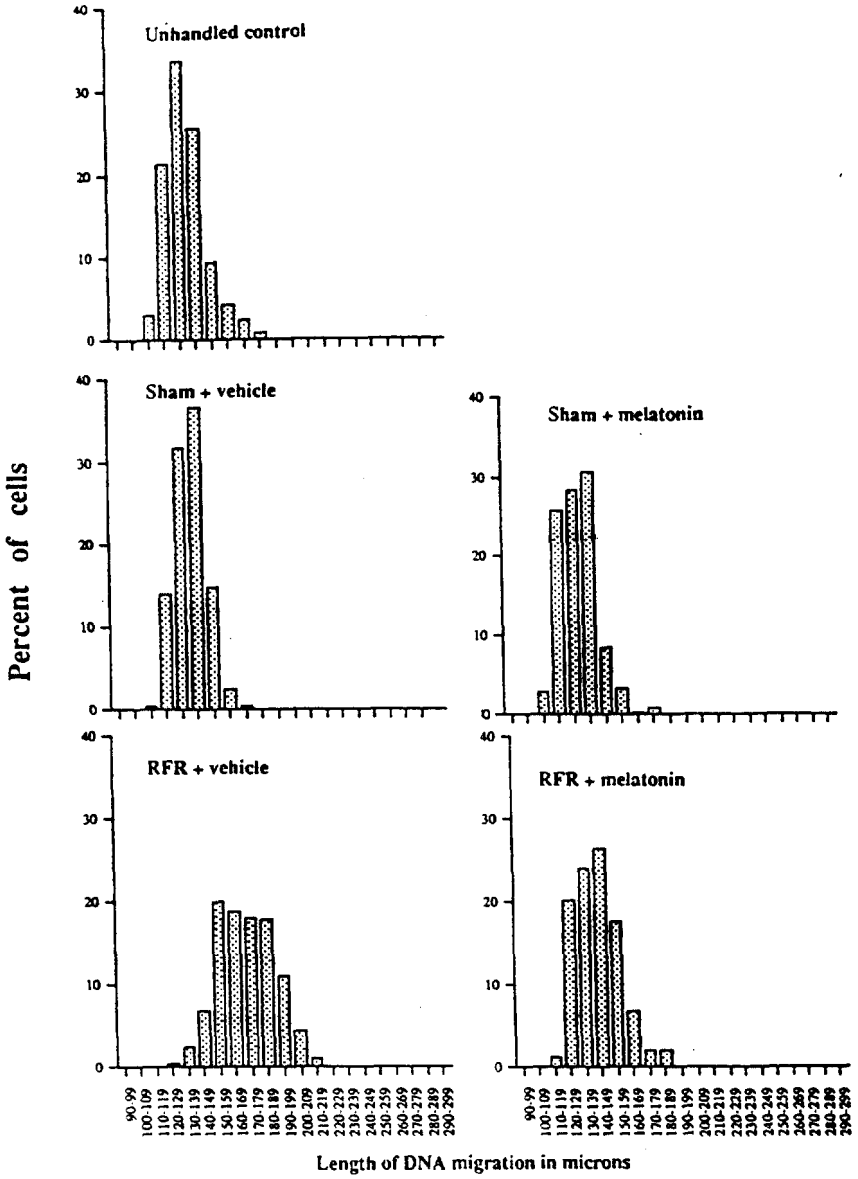

Fig. 4. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 3.

bination for fluorescence isothyocynate (excitation at 490 nm, emission filter at 515 nm, and dichromic filter at 500 nm). We measured the length of DNA migration (in microns) from the beginning of the nuclear area to the last 3 pixels of DNA perpendicular to the direction of migration at the leading edge. The migration length is used as the index of DNA strand breaks. In the present assay procedure, precipitation with ethanol enabled detection of smaller DNA fragments and increased the sensitivity and resolution of the assay. With this treatment, a significantly higher DNA migration length was detected. Without ethanol precipitation, the migration lengths of DNA from brain cells of a sham-exposed animal would be 40–50 microns.

Two slides were prepared from the brain sample of each animal: one for assay of single-strand DNA breaks, and the other for double-strand breaks. Fifty cells were randomly chosen and scored from each slide. However, cells that showed extensive damage, with DNA totally migrated out from the nuclear region, were not included in the measurement. These highly damaged cells probably resulted from the tissue and cell processing procedures. They occurred equally in RFR-exposed, sham-exposed, and unhandled samples.

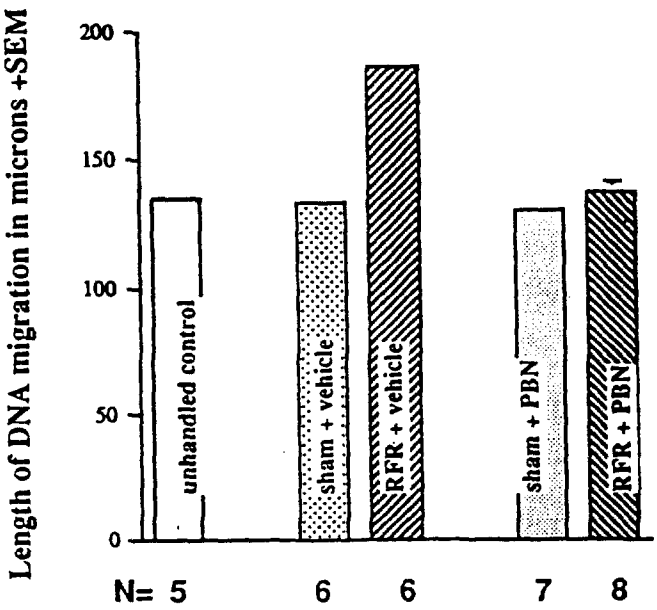

Fig. 5. Effect of treatment with PBN on RFR-induced increase in DNA single-strand breaks in rat brain cells. Data was analyzed using the one-way ANOVA, which showed a significant treatment effect ($F[4,27]= 75.5$, $P < .001$).

Therefore, from each animal, 50 cells each were scored for single- and double- strand DNA breaks.

Data Analysis

The average length of DNA migration from the 50 cells, measured for single- and double-strand breaks in each rat, was used in data analysis using the one-way ANOVA. The difference between the two treatment groups was compared by the Newman-Keuls Test with a difference of $P < .05$ considered statistically significant. Percentages of cells with respect to DNA migration length (in intervals of 10 microns) were also plotted.

RESULTS

Figure 1 shows the data of melatonin treatment on RFR-induced DNA single-strand breaks in brain cells of rats. RFR significantly increased DNA single-strand breaks in brain cells (RFR+ vehicle vs sham + vehicle; $P < .01$, Newman-Keuls test), whereas treatment with melatonin completely blocked the effect of RFR (i.e., no significant effect was found between RFR + melatonin and sham + melatonin). It should be pointed out that melatonin by itself has no significant effect on DNA single-strand breaks, i.e., no significant difference was found between the sham + melatonin and sham + vehicle groups. Experimental procedures also had no significant effect on DNA single-strand breaks in brain cells of the rat (i.e., there is no significant difference between the unhandled control and sham + vehicle groups). Percentage distributions of cells as a function of DNA migration length for the five treatment groups in this experiment are shown in Figure 2. Exposure to RFR caused a shift of distribution to longer lengths (i.e., to the right), and treatment with melatonin restored the distribution to a pattern similar to that of the sham + vehicle animals.

A similar conclusion can be drawn from data of the study on treatment with melatonin on DNA double-strand breaks in brain cells. Melatonin treatment blocked RFR-induced increase in DNA double-strand breaks in rats brain cells. Figures 3 and 4 plot the mean migration length and percent cell versus migration length distribution, respectively.

The results of treatment with PBN on RFR-induced increase in DNA single- and double-strand breaks in rat brain cells are presented in Figures 5–8. Similar to the effect of melatonin treatment, PBN blocked the RFR-induced increases in DNA single- and double-strand breaks in rat brain cells.

DISCUSSION

Data from the present experiment confirm our previous finding [Lai and Singh, 1995, 1996] that acute RFR exposure causes an increase in DNA single- and double-strand breaks in brain cells of the rat. In addition, we have found that the effect can be blocked by treating the animals with melatonin or PBN. Since a common property of melatonin and spin-trap compounds is that they are efficient free radical scavengers [Carney and Floyd, 1991; Carney et al., 1991; Floyd, 1991; Lafon-Cazal et al., 1993 a,b; Lai et al., 1986; Oliver et al., 1990; Reiter et al., 1995; Sen et al., 1994; Zhao et al., 1994], these data suggest that free radicals may play a role in the RFR-induced DNA single- and double-strand breaks observed in brain cells of the rat. Consistent with this hypothesis is the fact that free radicals can cause damage to DNA and other macromolecules in cells. Particularly, oxygen free radicals have been shown to cause DNA strand breaks [McCord and Fridovich, 1978]. In addition, a study has implicated free radicals as the cause of some of the biological effects observed after exposure to RFR. Phelan et al. [1992] reported that RFR can interact with melanin-containing cells and lead to changes in membrane fluidity consistent with a free radical effect.

If free radicals are involved in the RFR-induced DNA strand breaks in brain cells, results from the present study could have an important implication on the health effects of RFR exposure. Involvement of free radicals in human diseases, such as cancer and atherosclerosis, have been suggested. Free radicals also play an important role in aging processes [Reiter, 1995].

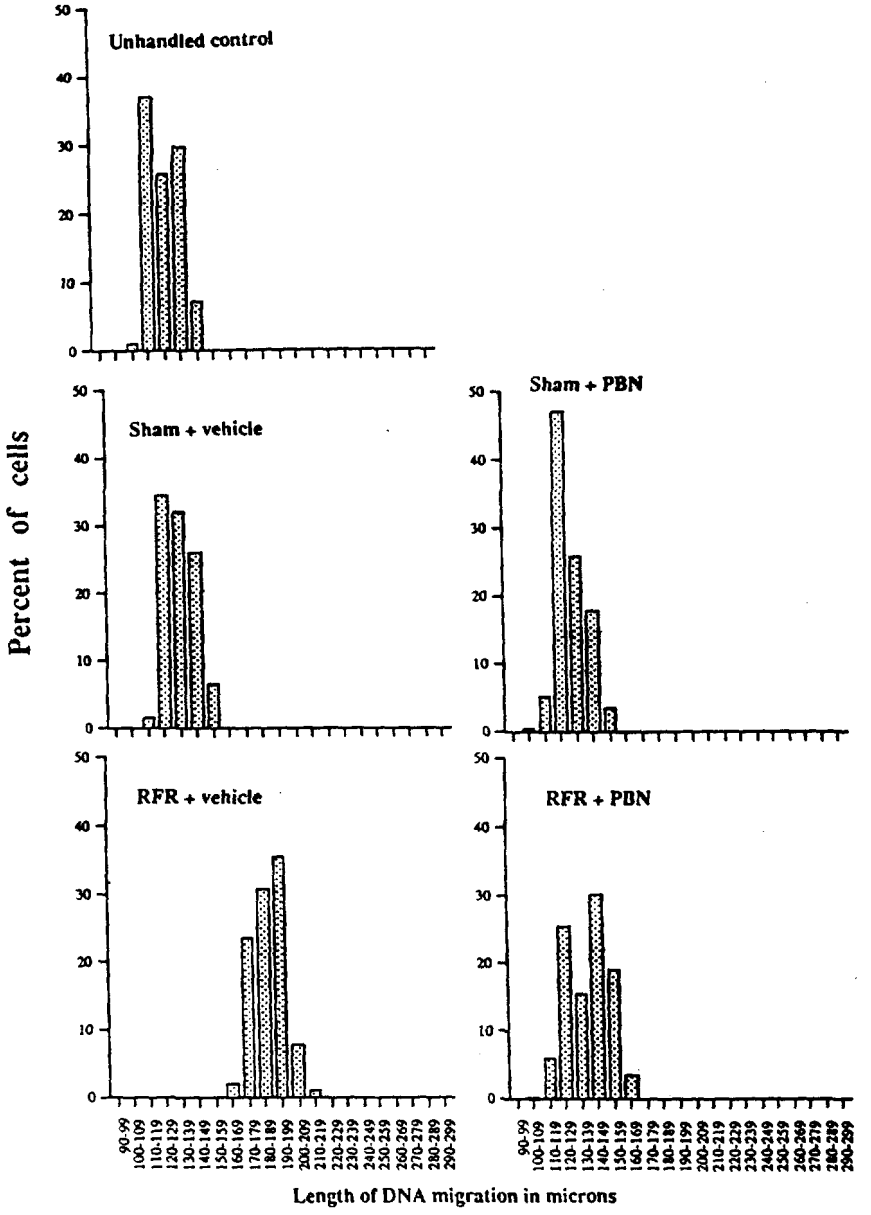

Fig. 6. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 5.

Aging has been ascribed to accumulated oxidative damage to body tissues [Forster et al., 1996; Sohal and Weindruch, 1996], and involvement of free radicals in neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's, has also been suggested [Borlongan et al., 1996; Owen et al., 1996]. Furthermore, the effect of free radicals can depend on the nutritional status of an individual, e.g., availability of dietary antioxidants [Aruoma, 1994], consumption of ethanol [Kurose et al., 1996], and dietary restriction [Wachsman, 1996]. Various life conditions, such as psychological stress [Hague et al., 1994] and strenuous physical exercise [Clarkson, 1995], have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can speculate that some individuals may be more susceptible to the effects of RFR exposure.

However, it must be pointed out that both melatonin and PBN can have other actions on cells in the brain that can ~~lead to~~ DNA damage. Further support for our hypothesis can be obtained by studying whether other compounds with free radical scavenging properties can similarly block the effect of RFR, and by measurement of other free radical-related cellular effects, such as oxidative molecular damages in lipids, protein, and DNA.

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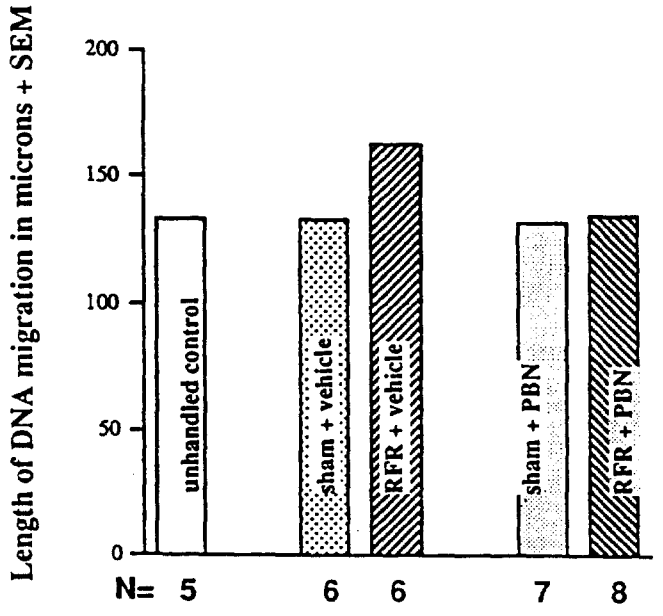

Fig. 7. Effect of treatment with PBN on RFR-induced increase in DNA double-strand breaks in rat brain cells. One-way ANOVA of the data showed a significant treatment effect ($F[4,27] = 47.83$, $P < .001$).

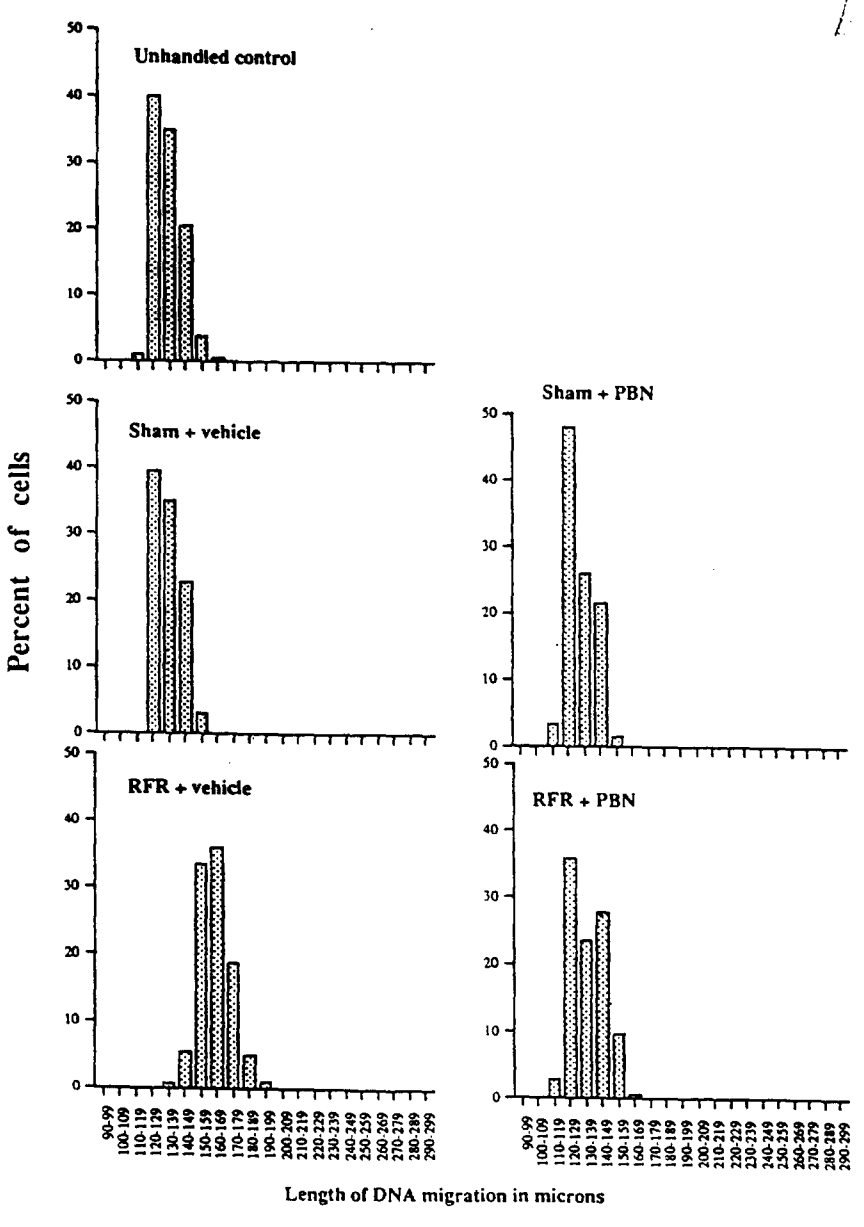

Fig. 8. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 7.

ACKNOWLEDGMENTS

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Original scientific paper

Comparison of chromosome aberration and micronucleus induction in human lymphocytes after occupational exposure to vinyl chloride monomer and microwave radiation

VERA GARAJ-VRHOVAC, ALEKSANDRA FUČIĆ and ĐURĐA HORVAT

Institute for Medical Research and Occupational Health, University of Zagreb, Moše Pijade 158, 41000 Zagreb, Yugoslavia

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SUMMARY. – Differences and similarities between the mutagenicity of microwave radiation and VCM were studied on the lymphocytes of persons occupationally exposed to either of these two clastogens. The results of the micronucleus test and the chromosome aberration assay were compared. Our findings provided evidence that the mutagenicity of both VCM and microwave radiation can be detected with both of these tests. However, we detected both quantitative and qualitative differences between VCM and microwave radiation in the induction of aberrations and micronuclei. VCM causes quantitatively more chromosome damage but less dicentric and ring chromosomes than microwave radiation. Anaphase bridges are constantly present in the cultured lymphocytes of VCM-exposed persons but they are rarely seen in the lymphocytes of persons exposed to microwave radiation. The number of micronuclei per a certain number of aberrations is much higher in personsd exposed to VCM than in those exposed to microwave radiation. An attempt was made here to point out the main differences in the clastogenic action of a physical and a chemical agent. Such data can be fundamental for better understanding of the genetic effects which derive from mutal action of chemical and physical clastogens.

INTRODUCTION

Chromosomal damage which results from exposure to a wide range of chemical or physical agents is associated with severe health disorders. Together with the conventional chromosome aberration assay, the cytochalasin B method - technique, which is based on the analysis of micronuclei in cells whose cytokinesis has been blocked with cytochalasin B can be used for mutagenic assessment *(12)*.

Immunofluorescence techinques and complex mathematical methods have shown that there is no simple relationship between acentric fragments and the apperance of micronuclei *(6, 16, 31)*. The mechanism leading to chromosome damage by physical agents and chemical substances are different. Today there are several studies which confirm the mutagenicity of non-ionizing radiation. The hypothesis explaining that microwave radiation as ultrasound acts by mechanical disruption of DNA and by free radicals which result in one-strand or double-strand breaks of the DNA molecule has not yet been confirmed *(21, 22, 29)*. Vinylchloride monomer (VCM) is a well known clastogen which causes chromosomal damage through its metabolites in persons exposed to high levels of this chemical *(1, 2, 9, 13, 14, 15, 27, 32)*

The aim of our study was to compare the results of the lymphocyte micronucleus test and the chromosome aberration assay from subjects exposed to VCM or (alternatively) to microwave radiation. The correlation between the mutagenic effects of these two clastogens has been discussed.

TABLE 1

Comparison of percentage of micronucleated cells and aberration types in persons exposed to microwave radiation.

Case No.	CELLS WITH MICRONUCLEI (%)					CELLS WITH ABERRATIONS (%)						
	No. of micronuclei in the cells				Total %	Anaphase bridge	Chromatid break	Chromosome break	Acentric	Dicentric	Ring	Total %
	0	1	2	3								
1	98.40	1.60	0	0	1.60	0.40	0	0.34	1.37	0	0	1.61
2	97.40	2.60	0	0	2.60	0	0	1.20	0.80	0	0	2.00
3	96.50	3.50	0	0	3.50	0	0.60	1.81	3.03	0	0	5.44**
4	96.04	3.50	0.36	0	3.06	0	0.69	4.19	0.69	0	0	5.57**
5	96.00	4.00	0	0	4.00	0	0.68	2.06	1.38	0	0.34	4.46**
6	95.10	4.90	0	0	4.90*	0.40	2.50	0	2.50	0	0	5.00**
7	95.00	4.60	0.40	0	5.00**	0	1.00	1.00	0	0	0	2.00
8	91.20	7.80	1.00	0	8.80***	1.00	0	3.30	6.60	3.30	0	13.20***
9	90.30	8.50	0.60	0.60	9.70***	0	0.70	2.11	2.81	0.70	0	6.62***
10	72.10	25.20	2.70	0	27.90***	0.90	10.50	0	21.00	0	0	31.50***
Controls	98.20	1.80	0	0	1.80	0	1.00	0.50	0.50	0	0	1.50

*Statistically significant increase compared with the controls: *$p<0.05$, **$p<0.01$, ***$p<0.001$.*
For all calculations 1000 cells for micronucleus test and 500 cells for chromosome aberration assays was counted per persons.

MATERIAL AND METHODS

Subjects

Ten workers from a radar station service and ten workers from the polyvinylchloride industry were randomly chosen for the study. The workers, in both cases had been employed for 15 years (ranging from 8 to 25 years), on an average. Persons who had not been occupationally exposed to any known mutagenetic agent were chosen as controls. The power density of microwave radiation was measured at different vorking places of examinees with the Raham, model 4A, which measures the frequency range from 0.2 MHz to 26 GHz, power range being between 0 and 20 mW/cm^2. At the working places the power density of the electromagnetic field varied between 10 μW/cm^2 and 50 μW/cm^2. The VCM concentration in the working environment was an average of, 5 ppm. Due to the technological process, the concentration periodically reached higher values during a short period of time.

Culture and analysis of the cells

Two parallel whole blood cultures from persons occupationally exposed to VCM and microwave radiation were established for analysis of chromosome aberrations and micronuclei in lymphocytes.

The chromosome aberration assay was performed from the cultures of phytohemaglutinin stimulated lymphocytes. The cultures contained 0.5 ml whole blood, 8 ml F-10 media (GIBCO) containing 20% calf serum. The cells were incubated at 37°C for 48 hours. After 45 hrs colchicine was added. Fixation of the cultures and preparation of slides was carried out according to the conventional method *(5)*.

500 cells with well spread metphases per person were analysed for chromosome aberrations which were classified as acentric fragments, dicentrics, rings, and chromosome and chromatid breaks.

For the micronucleus preparations cytochalasin B, at a final concentration of 3 μg/ml, was added at 44 h of the culture according to the method of Fenech and Morley *(12)*. Micronucleus slides were made by fixation in 3:1 methnol : acetic acid without hypotonic treatment, dropping the cell sspension onto clean cold slides and by staining in Giemsa. 1000 cells per person were analysed for micronuclei. The results are presented as a percentage distribution of cells with 1, 2, 3 and 4 micronuclei and as percentage of anphase bridges. The results (Tables 1, 2 and 3) were analysed statistically using the chi-square method *(26)*.

RESULTS

The incidence of micronuclei and chromosome aberrations in the microwave radiation and VCM – exposed subjects for ten donors are shown in Tables 1 and 2. Elevated levels of

TABLE 2

Comparison of percentage of micronucleated cells and aberration types in persons exposed to vinyl chloride monomer.

Case No.	CELLS WITH MICRONUCLEI (%)						CELLS WITH ABERRATIONS (%)						
	No. of micronuclei in the cells					Total %	Anaphase bridge	Chromatid break	Chromosome break	Acentric	Dicentric	Translocation	Total %
	0	1	2	3	4								
1	97.90	2.10	0	0	0	2.10	0	2.00	1.00	0.50	0	0	3.50
2	96.40	3.60	0	0	0	3.60	0.71	2.50	1.00	1.50	0	0	5.00*
3	95.00	5.00	0	0	0	5.00*	0.40	3.00	1.50	0	0	0	5.50*
4	93.50	6.50	0.36	0	0	6.50*	1.00	6.00	0	1.00	0	0.50	7.00**
5	89.70	10.30	0	0	0	10.30***	1.03	5.50	1.00	0.50	0	0	9.00***
6	89.50	10.50	0	0	0	10.50***	1.75	5.50	2.00	1.50	0	0	9.00***
7	88.70	9.00	0.40	0	0	11.30***	0.75	6.00	0.50	2.00	0	0	8.50***
8	87.70	5.40	1.00	0	0	12.30***	0.70	7.00	0.50	1.00	0.50	0.50	9.50***
9	85.00	13.60	0.60	0	0	15.00***	0.70	8.00	1.00	1.50	0.50	1.00	12.00***
10	81.82	16.80	2.70	0.37	0.37	12.28***	1.12	6.50	0	0.50	0.50	1.50	9.00***
Controls	98.20	1.80	0	0	0	1.80	0	1.00	0.50	0.50	0	0	1.50

*Statistically significant increase compared with the controls: *$p<0.05$, **$p<0.01$, ***$p<0.001$.*
For all calculations 1000 cells for micronucleus test and 500 cells for chromosome aberration assays was counted per persons.

micronuclei and chromosome aberrations by both clastogenic agents were observed. For comparison the tables contain the frequency of micronuclei and chromosome aberrations in the control group. The percentage of micronuclei in controls is very low and reaches the value of 1.8% and for chromosome aberrations 1.5%. Only acentric fragments, chromosome breaks and chromatid breaks were observed in the controls.

The highest individual micronucleus value in peripheral lymphocytes after exposure to vinylchloride monomer was 18.3% and for the chromosome aberrations it was 12%. Those exposed to microwave radiation showed the highest level of 27.9% for micronuclei and 31.5% for chromosome aberrations. Persons exposed to microwave radiation showed a much higher frequency of acentric fragments, dicentrics and ring chromosomes compared to those exposed to vinylochloride monomer.

From Figures 1 and 2 it can be concluded that the two parameters increased in parallel.

Both microwave radiation and VCM increased the number of cells with more than one micronucleus (Tables 1 and 2). Both agents also induced cells with multiple chromosome aberrations. In these cells the chromosomes were more severely damaged than in cells which contained few aberrations.

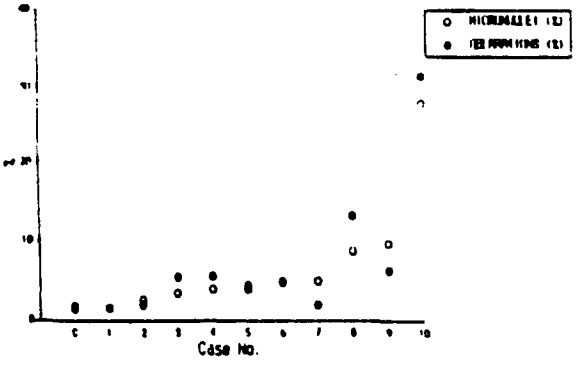

FIGURE 1. *Percentage of cells with micronuclei and total aberrations in persons occupationally exposed to microwave radiation and in the control persons (C).*

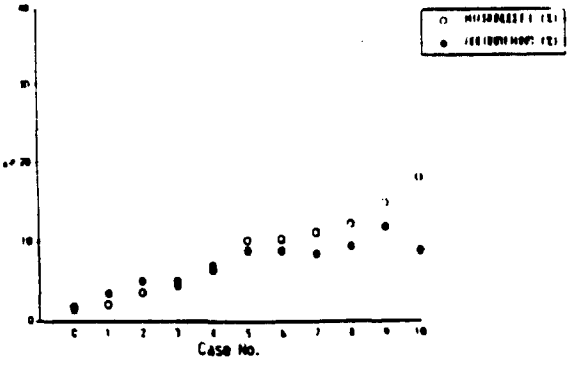

FIGURE 2. *Percentage of cells with micronuclei and total aberrations in persons occupationally exposed to vinyl chloride monomer and in the control persons (C).*

TABLE 3

Ratio between percentages of cells with micronuclei and each type of chromosome aberrations after action of a physical and a chemical agent.

Aberration type	CELLS (%) WITH MICRONUCLEI/ABERRATIONS exposure to microwave radiation	exposure to VCM	controls
Chromatid break	4.3	1.8	1.00
Chromosome break	4.5	11.1	0.50
Acentric	1.8	9.5	0.50
Dicentric	18.0	63.2	0

The percentage of anaphase bridges is much higher in persons exposed to VCM, than in subjects exposed to microwave radiation.

The numbers of chromosome aberrations and micronuclei are significantly ($p<0.05$, $p<0.01$, $p<0.001$) higher in the exposed workers than in the controls. The increase (in the workers) is more evident in the case of structural chromosome aberrations as to both agents.

DISCUSSION

Differences and similarities between the mutagenicity of physical and chemical agent have been poorly investigated. In our study we used the micronucleus and chromosome aberration assay of peripheral lymphocytes to compare the mutagenic actions of microwave radiation and VCM.

The mechanism of the genotoxicity of microwave radiation and VCM are different. VCM is metabolized into mutagenic intermediates by the liver microsomal fraction in the presence of an NADPH-generating system *(4)*. Mutagenic intermediates of VCM, such as chloracetaldehyde lead to depurinification of DNA. During the repair process there is a risk that such a gap will be substituted unappropriately. Non-inonizing radiation on the other hand, may act through cavitation. The process of cavitation represent, at the same time, mechanical action which results in a hydrodynamic breakage of hydrogen bonds and chemical action by free radicals *(3, 17, 21, 23)*. The lesion in DNA molecule, induced, by the mechanical effect of e. g. ultrasound seems to be a double-strand break. The majority of single-strand breaks induced by ultrasound are caused by active radicals originating from cavitation *(21)*.

Data on the induction of micronuclei by non-ionizing radiation or vinylchlorid monomer are poor. Few experiments have been done with animals which discuss the change in the number of micronuclei in bone-marrow cells after exposure either to electric fields or vinylchlorid monomer *(18, 25, 28)*. The results of studies on occupationally exposed workers agree with our results here. The present observations provide evidence that the mutagenicity of VCM and microwave radiation can be detected not only by the chromosome aberration assay but also with the micronucleus test.

The percentage of micronuclei in microwave-exposed persons ranged from 1.6% to 27.9% and for the chromosome aberrations from 1.6% to 31.5%. In the case of VCM exposure the percentage of micronuclei ranged from 2.1% to 18.3% and for the aberration from 3.5% to 12%. The chromosome aberration test showed a more significant increase compared to the micronucleus test (Tables 1 and 2), even though the results of these cytogenetic parameters were parallel.

The total number of aberrations caused by VCM was higher than that caused by microwave radiation but the latter agent caused more acentric fragments and dicentrics than VCM. In Table 3, the ratio between the frequency of cells with micronuclei and aberrations indicate that the increased number of micronuclei caused by VCM is a result of numerous but less severe chromosome aberrations compared to the effects induced by microwave radiation.

This is in agreement with the observations of Kihlman *(19)* who has already found that chemical agents produce mainly aberrations of the chromatid type which is in sharp distinction to the results of treatment with radiation which induces also aberrations of the chromosome type.

The anaphase bridges are not recommended for measuring cytological damage because their number depends on many factors *(7, 8, 11)*. It is important to mention however that anaphase bridges were constantly present in the VCM exposed workers, but rarely seen in workers exposed to microwave radiation.

The percentage of micronuclei indicates clastogenicity and chromosome lagging which leads to aneuploidy. These effects may be associated with the induction of tumors or neoplastic transformations *(20, 24, 30)*. Accordingly, the micronucleus assay may be extremly valuable for monitoring the loss of genetic material and clastogenic exposures.